Carboxylesterases: Sources, Characterization and Broader Applications

Abstract
Carboxylesterases (CEs) are a group of versatile lipolytic enzymes capable of catalyzing the hydrolysis of esters into acid and alcohol molecules. These enzymes are extensively used in diverse xenobiotic and endobiotic degradations, biocatalysis, and drug metabolism. The present review article focuses on structure, function and major applications of CEs mainly sourced from bacteria and archaea. The CEs are divided in different families (15 families) depending upon their source, biochemical properties, common pentapeptide motif (GXSXG) with catalytic Ser, an entirely different GDSL motif, (family II)/ SXKK motif (family VIII), position of catalytic triad and their protein forms. CEs find diverse applications in degradation of xenobiotic compounds, biocatalysis, biotransformation of compounds such as cholesterol, synthesis of optically active compounds, food industry, anticancer therapeutics, drug and prodrugs like aspirin, delapril etc.

Keywords: Carboxylesterases (CEs); Structure; Function; Families; Mechanism; Properties; Distribution

Introduction
Esterases, particularly carboxylesterases (CEs) belong to the family of serine esterases, which hydrolyze simple esters (ethyl acetate) and triglycerides of short chain length (C ≤ 6) [1]. These enzymes catalyze the hydrolysis of short-chain aliphatic as well as aromatic carboxylic ester compounds and are often inhibited by small concentration of organophosphate (OP) compounds [2]. Esterase-catalyzed reactions are mostly specific for an alcohol or acid moiety of the substrate but not for both. A carboxylesterase from Pyrobacculum calidifontis VA-1 has a strong affinity for an alcohol moiety [3,4]. Structurally and functionally these enzymes are similar to the other members of α/β hydrolase fold superfamily, but have less amino acid sequence identity with other members of this family [5]. However, these enzymes are multifunctional in nature. A single esterolytic reaction can catalyze the hydrolysis of esters as well as amides. As in signature amidases which contain conserved amino acid sequence of 130 amino acids rich in Gly (G) and Ser (S) but lack Asp (D) and His (H) that are typical characteristic of carboxylesterases. The substrate affinity for substrates like ethyl acetate, ethyl butyrate, tributyrin as well as phenyl acetate classify carboxyl ester hydrolases into acetylesterases, arylesterases and carboxylesterases. The substrate specificity of CEs also divides them into two categories; specific and nonspecific. Being adapted to different functions some CEs have strong substrate selectivity (e.g., acetylcholinesterases) whereas others that are less selective and are referred as true esterases [5,6]. Carboxylesterases have broad substrate specificity due to large conformable active site, which allows structurally diverse substrates to sit at it. The CEs have been classified on the basis of sequence similarity into eight families [7] but later on classification extended and their number increased. Their broad substrate specificity enables them to be involved in the evolution of carbon sources and diverse catabolic pathways [8]. Many people classified them differently based on their insecticide resistance into EstA, EstB and EstC [9]. Because of their inhibition by organophosphate compounds (OP’s), CEs are defined as EstB. The chromophoric substances e.g., para-nitrophenyl esters or tributyrin-supplemented agar plates are commonly used for the screening of microbial CEs. Active
esterase-producing organisms form clear halos in agar Petri plates containing soluble substrates such as triglycerides. Hydrolysis of these dispersed lipids results in the formation of clearing zones around the bacterial colonies [10]. Enzyme assays to determine the esterase activity include spectrophotometric and saponification methods [11]. These enzymes help to metabolize drug esters and amides carbamates [12].

### Carboxylesterases sources

CEs have been isolated from diverse sources including bacteria, fungi, algae, animals, plants and human beings. A number of carboxylesterase genes have been cloned and expressed in suitable host systems. They are present in all environments including moderate and extreme temperature range habitats which shape these enzymes. CEs have also been reported from cold- and salt-resistant marine microorganisms. The hyperthermophilic microbial sources mostly include Archaeabacteria. The first CE was reported from a thermo-acidophilic bacterium *Sulfolobus acidocaldarius* and its 3D structure was also elucidated. Another, esterase elucidated from genus *Alkalibacterium* sp. was the first reported esterase from halophilic cold-adapted environment [13].

### Metagenomic sources

Metagenomic samples are quite commonly used for the isolation of CEs. Most of the esterases have been isolated by metagenomics approach which is a cultureless method [14,15]. It is a common technique to explore novel enzymes that have potential for industrial applications. The EstCS2 isolated from metagenomic samples can hydrolyze (R/S-ketoprofen ethylester) with great enantioselectivity for R-enantiomer of it. Ketoprofenethylester is a non-steroidal anti-inflammatory drug (NSAID) having analgesic, antipyretic and anti-inflammatory affects as well as great enantioselectivity for R-enantiomer [16]. Insect-derived CEs are categorized into α-esterases, β-esterases, juvenile hormone esterase, gleotactins, neurotactins, neuroligins and glutactin classes.

### Bacterial sources

Because of the ease with which bacterial cells may be mass cultured and genetically manipulated, the bacterial strains are considered superior sources of these enzymes than the higher organisms. However, CEs have also been isolated from the higher animals like mammals. The genomes from most hyperthermophilic archaeaons have been well characterized and have been completely sequenced too, e.g., *Archaeoglobus fulgidus* [17].

### Human carboxylesterases

In human, CEs (hCEs) these are present in the form of isozymes. They are present in different organs in human body like brain, liver, kidneys, muscles and adipose tissue. Within a cell, the carboxylesterases are confined to endoplasmic reticulum. Human genome organization database listed five types of carboxylesterase genes family CES1, CES2, CES3, CES4, and CES5 [18]. An additional CES6 gene family also includes structures of many mammalian carboxylesterase [19]. In mammals, the first crystal structure was deciphered for rabbit liver carboxylesterase (rCE). Its structure bears 81% sequence identity with hCE-1.

### General Structure of CEs

The amino acid sequence of CEs belongs to superfamily of α/β hydrolase enzymes. The structure of the enzyme consists of central eight stranded β-sheets surrounded by helices and connecting loops. The catalytic structure of these enzymes is made up of Ser (S), His (H) and Asp (D). Ser (S) is generally found fixed in a pentapeptide motif (GXSGX) where X is any residue. Nucleophelic elbow is a sharp turn present in the 3D structure and is produced by this motif. This nucleophelic elbow is located at the apex of sharp turn between α-helix and β-sheet. The structure of CE enzyme was first determined from *Pseudomonas fluorescens*. Use of serine protease inhibitor, phenylmethylsulphonyl fluoride (PMSF) and isomorphous replacement method revealed the CE-catalytic triad comprising Ser-114, His-199 and Asp-168 for first time within the structure of *Pseudomonas fluorescens*. An open active site and a large binding pocket for acid moiety of substrate contribute towards the broad substrate specificity of CE [20]. The backbone carbonyl of the residue was located three residues upward from His (H), and it becomes an important factor to stabilize the His (H) positive side-chain instead of the negative moiety of Asp (D) in the catalytic triad [7,21].

### Mechanism of Action of CEs

Esterases catalyze different types of reactions. The primary reaction catalyzed by these enzymes is hydrolysis, but depending upon the reaction conditions, CEs also catalyze esterification, alcoholysis, and transesterification reactions.

The ability of these enzymes to hydrolyze ester bond to acid and alcohol metabolite is a two-steps process (Figure 1). The first step is nucleophilic attack of catalytic serine –OH on the carbonyl carbon of ester bond. This step releases an alcohol metabolite due to covalent linkages formed between acid moiety of substrate and Ser (S) residue at the catalytic site. The step is stabilized through hydrogen bonding to His (H) which in turn is stabilized by carboxylic group of acidic member of catalytic triad. In the second step, the His (H) residue of the catalytic triad shows affinity toward water molecules and it also helps the enzyme to return to its active state with the release of an acid moiety.

![Figure 1](http://www.imedpub.com/insights-in-enzyme-research/)
Enzymatic Properties of Carboxylesterases

Among all the enzymes, CEs are the only enzymes which can bear the harsh conditions of industrial biotransformation like high temperature, broad pH range and exposure to denaturing organic solvents. The CEs sourced from natural solvents or microbes are ecologically friendly and those of thermophilic microbial origin have a natural advantage to perform biocatalysis at temperatures above ambient, too.

Hydrolysis of Water Soluble Ester Substrate

The CEs often show inherent specificity towards the substrates of short to moderate chain lengths (C ≤ 12) whereas substrates of longer chain length are poor substrates for these enzymes. Tributyrin (C₆H₁₄O₂₃) is a standard substrate to assess the CE activity. The substrate specificity of CEs shows p-nitrophenybutyrate being cleaved by carboxylesterases. Their affinity for short and medium chain length esters is relatively much more than the long carbon chain length esters. This is a criterion employed for their demarcation from lipases.

CEs do not act on the substrates that form micelles

CEs do not appear to act at interface created by hydrophobic lipid substrate(s) and hydrophilic aqueous medium called interfacial activation. However, some of the scientists are of the opinion that the phenomenon of interfacial activation is not a sole distinguishing character between lipases and CEs; e.g., interfacial activation is absent in lipase Lip A from Bacillus subtilis though lid is absent around the active site whereas it is present in lipase Lip A from Pseudomonas aeruginosa [22]. Further the CEs obey Michaelis-Menten equation i.e., these enzymes require minimum substrate concentration for a reaction to occur.

Biochemical Properties of Bacterial Carboxylesterases

Molecular mass of CEs

The most of CEs exist as monomer and oligomers of same subunits with molecular weight broadly ranging from 25 kDa to 85 kDa.

Temperature and pH optima

The CEs show activity in the buffer system with pH raging from alkaline to acidic (pH 3-13) but appear to show maximum activity at pH 7 or 8 i.e., either at neutral or alkaline pH.

Thermophilic, mesophilic and psychrophilic CEs

Bacterial CEs are present at all temperature conditions from moderate to extreme. This enzyme works in the temperature range of 35-65°C but its optimum temperature intriguingly lies at both low as well as high temperature. Highly thermostable CE from hyperthermophilic Pyrobaculum calidifontis VA-1 has a temperature optimum at 90°C while an esterase from Pyrococcus furiosus has half-life period of 2 hours at 110°C [4]. Most of hyperthermophilic microbes including archaeal sources have been characterized and have completely sequenced genomes.

However, very few CEs have been sequenced and characterized from hyperthermophilies sources. The first 3D structure CE that was successfully characterized was from an acidothermophilic bacterium Sulfolobus acidocaldarius [23-24]. CEs also show their presence in halophilic environment [25]. An esterase EstSL3 from Alkalibacterium sp. was the first esterase reported from a cold-adapted salted environment of a soda-lake which was capable of functioning both at high pH and high salt concentration. Such enzymes show adaptation to highly cold environment [13].

Enzyme assays and substrate specificity

The activity of CEs can be measured in terms of a Unit (U) which refers to the amount of enzyme that releases 1 µmol of para-nitrophenol from a para-nitrophenylester (often para-nitrophenyesters of C-chain length ≤ 12) per min under specified conditions of temperature and pH [26-28]. Lowering of enzyme activity with the increase in fatty acid chain length is the characteristic of true esterases.

Effect of metal ions on the activity of CEs

To maintain enzyme stability and active enzyme protein structure, divalent metal ions often play an important role. Among divalent cations of Ca, Mg, Mn, Fe, Co, Cu, Cd and Hg, most of these have inhibitory effect on the activity of the esterases, whereas some of these might have stimulatory effect, too. In case of some esterases, the Ca²⁺, Cd²⁺ and Mg²⁺ ions result in better binding of enzyme on to the substrate molecules and also the neutralisation of fatty acid released from the substrate [29]. In oil-degrading bacterium Acinetobacter Iwoffii 16C-1, the addition of divalent cations decreased the activity to greater than 50% resulting in the binding of free fatty acids onto the oil surface thereby increasing their surface area [30]. Some esterases such as PyTH pyrrothroid-degrading CE from Sphingobium sp. JZ-1 showed little role of metal ions in enzyme activity due to its inactivity towards EDTA, a chelating agent [31].

Carboxylesterase inhibitors

The structure and catalytic properties of carboxylesterases are understood by the use of a variety of inhibitors. Inhibitors significantly affected the discovery of anti-esterase drugs (Table 1). The activity of these enzymes is greatly affected by various inhibitors. PMSF, a serine protease inhibitor can covalently link to serine, while diisopropylfluorophosphate (DIFP) and diethylpyrocarbonate (DEPC) are commonly used inhibitor, which inhibit esterase activity thus inferring that Ser (S) as well as His (H) residues are indiscernible for the activity of these enzymes. Change in CEs activity alters drug metabolism and pharmacokinetics and also results in the development of small molecular inhibitors with the purpose of altering drug-induced toxicity. CE inhibitors significantly affected the discovery of drugs [32].

Effects of detergents and organic solvents on CEs

Esterases due to their inherent stability in organic solvents are of immense use in industrial biotechnology-based application. The ability of these enzymes to reverse the reaction from hydrolysis to synthesis by the removal of water makes them beneficial.
in various commercial esterification reactions. An esterase from *Lactobacillus plantarum*, a lactic acid bacterium which is involved in fermented food products shows resistance to organic solvents and metal cations [33]. The detergents being surface-active molecules may enhance the spreading/insufficiency of the substrate molecules leading to improved activity of CEs [34].

### Classification of CEs

Esterases may be classified based on their substrate specificity (Table 1) as well as their catalytic inhibition pattern (Tables 2 and 3). This classification scheme although did not exactly classify CEs yet it helps to determine biochemical properties of the esterases. In the case of *Sulfobolus solfataricus*, some inhibitors like PMSF reduces the activity of the enzyme which means that the serine residue(s) at the active site of the enzyme is/are involved while the inhibition of CE-activity by mercuric chloride indicates the presence of sulphydryl (-SH) group, too [35].

Based upon the specificity of the enzyme for its substrate (chain length), GDSL motif, triad nature of catalytic site, nucelophilic serine and consensus sequences, the esterases have been classified into I to XV families (Table 3).

#### Family I

The family I contains true lipases and this family is further subdivided into six subfamilies.

#### Family II

The CEs in this family show GDSL motif instead of classical GXSG present in most of these esterases. These enzymes have been reported from *Streptomyces scabies*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Ferribidobacterium nodusum* Rt 17 B-1 and an esterase EstSL3 from *Alkalibacterium* sp. [43]. The esterase from *Streptomyces scabies* lacked Asp (D) and contained a diad of Ser (S) and His (H), instead of a typical catalytic triad is a member of this family. The backbone carbonyl of the residue located three residues upward from His (H) become a stabilizing factor to stabilize His (H) positive side chain instead of negative chain of Asp (D) in catalytic triad [7,21]. GDSL family is further subdivided based on conservation of S, G, H and D residues into four blocks I, II, III and IV. The enzymes in this family possess different functional properties and broad substrate specificity due to flexible active catalytic site that undergoes induced-fit conformational change [44,45].

#### Family III

These CEs are characterized by a triad Ser (S), Asp (D) and His (H) which have functional resemblance with subtilisin and trypsin. The family consists of majority of lipid hydrolyzing CEs. MtEst4S from *Microbulbifer thermotolerans* DAU221 from this family is categorized as an esterase although its substrate kinetics shows maximum activity with carbon chain length of 4 and poor activity with C chain length of 2 and 6 [29].

#### Family IV

These esterases are called hormone-sensitive lipases because of their primary amino acid sequence shows similarity with that of hormone sensitive lipases. The active site Ser (S) is the part of conserved sequence pentapeptide motif GDSLG at position Ser-157, His-254 and Asp-284 and an oxyanion hole having sequence motif HGGG F/W/Y, which are involved in hydrogen bonding interactions and also stabilize the transition state during the hydrolysis. There is a distinct sequence similarity between esterases from mesophilic, thermophilic and psychrophilic CEs. Some of the esterases at thermophilic and extreme ends from this family are *Archaeoglobus fulgidus* [46], *Sulfobolus solfataricus* P1 [47], *Pyrobacculum calidifontis* [4], *Bacillus acidocaldarius* [48], Est ATII; as well as halotolerant forms whereas its mesophilic counterparts are *Lactobacillus plantarum* [49], *Oenococcus oeni* [50], *Acinetobacter lwoffii* 16 C-1 [30], and some members are also isolated from metagenomic sources [14,51,52]. The metalloresistant Est ATII is a thermophilic as well as halotolerant esterase reported from extremely harsh conditions [53]. Est J was the first alkaliphilic and moderately thermophilic esterase and first thermoalklostable 499EST esterase has been reported from gram negative bacterium *Acidicallidus* sp. strain USBA-GBX-499 [54]. Proteins present in this family are monomers and dimers as in Pest E from *Pyrobacculum calidifontis* VA-1 and octamers also as is the case of esterase AFest from *Archaeoglobus fulgidus*. The active site as well as substrate binding site is complete within monomer. All HSL proteins in their structural form show same processive orientation of substrate in the active site [55]. An esterase from *Pyrobacculum calidifontis* VA-1 is the most stable thermophilic esterase among all the esterases reported from this family. This esterase can sustain its activity for 2 h at 100°C.

### Table 1: Bacterial carboxylesterases and their substrate specificities.

<table>
<thead>
<tr>
<th>Substrates</th>
<th>CE producing microorganisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Phenyl acetate ($C_{10}H_{12}O_2$)</td>
<td><em>Rhodobacter sphearoideis</em></td>
</tr>
<tr>
<td>4-Nitrophenylacetate ($C_7H_6NO_2$)</td>
<td><em>Bacillus subtilis</em>, <em>Geobacillus stereothermophilus</em>, <em>Pseudomonas fluorescens</em>, <em>Arthrobacter globiformis</em>, <em>Archeoglobus fulgidus</em>, <em>Alicyclobacillus acidocaldarius</em>, <em>Lactobacillus caseae</em>, <em>Klebsiella sp.</em>, <em>Sulfobolus solfataricus</em>, <em>Serratia sp.</em>.</td>
</tr>
<tr>
<td>2-Naphy acetate ($C_{12}H_{16}O_2$)</td>
<td><em>Pseudomonas fluorescens</em>, <em>Rhodobacter sphearoideis</em>, <em>Sulfobolus solfataricus</em>, <em>Thalasobacillus sp.</em>.</td>
</tr>
<tr>
<td>2-Naphyl butyrate ($C_{13}H_{22}O_2$)</td>
<td><em>E. coli</em></td>
</tr>
<tr>
<td>2-Naphyl propionate ($C_{12}H_{16}O_2$)</td>
<td><em>Rhodobacter sphearoideis</em></td>
</tr>
<tr>
<td>Ethyl chrysanthenate ($C_{12}H_{14}O_2$)</td>
<td><em>Arthrobacter globiformis</em></td>
</tr>
<tr>
<td>Isopropylidine glycerolbutanoate ($C_{12}H_{20}O_2$)</td>
<td><em>Bacillus subtilis</em></td>
</tr>
<tr>
<td>Ketoprofen ethylester ($C_{12}H_{14}O_2$)</td>
<td><em>Archeoglobus fulgidus</em>, <em>Pseudomonas fluorescens</em></td>
</tr>
<tr>
<td>Naproxen methyl ester ($C_{14}H_{20}O_2$)</td>
<td><em>Bacillus subtilis</em>, <em>Sulfobolus solfataricus</em> and <em>Sulfobolus solfataricus</em> P1</td>
</tr>
</tbody>
</table>
Family V

The enzymes in this family originate from mesophilic bacteria such as Acetobacter pasteurianus, Pseudomonas oleororans and Haemophilus influenza as well as from cold-adapted Sulfolobus acidocaldarius. Only a few esterases have been characterized from this family.

Family VI

These esterases are smallest known esterases having molecular weight of 23-26 kDa. The crystal structure of an esterase from Pseudomonas fluorescense has been successfully determined [20]. Est A6 from a Pseudomonas sp. CR-611 [56] belonged to this family. A pentapeptide motif GXSXG with catalytic triad of Ser-Asp-His hydrolysing small substrates is present in these esterases. CES in this family are present as active homodimeric proteins. Highly alkal tolerant, halotolerant and enatioselective Est PEB from marine Pelagebacterium halotolerans B2T is a potential source of a precursor (R)-3-MFG used for the synthesis of anti-depressant paroxetine hydrochloride. Among all the esterases, this family is known for its smallest (23-26 kDa) molecular mass [57]. Another esterase from Spirulina platensis [58] and pyrethroid hydrolysing esterase from Ochrobactrum anthropi YZ-1 have also been reported from this family [59].

Table 2 Classification given by different researchers.

<table>
<thead>
<tr>
<th>S No</th>
<th>Criteria of classification</th>
<th>Types of esterases</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Use of inhibition pattern such as organophosphates paraoxon diisopropyl fluorophosphate, fenitrooxon, Carbamate esterine sulphate</td>
<td>a) Acetyesterases not affected by inhibitors and prefer aliphatic substrates b) Arylesterases prefer aromatic substrates and inhibited by sulphhydryl reagents. c) Carboxylesterases inhibited by organophosphates (OP) prefer aliphatic substrates d) Cholinesterase simultaneously inhibited by OP and eserine sulphate.</td>
<td>[5]</td>
</tr>
<tr>
<td>2.</td>
<td>International Union of Biochemistry and Molecular Biology (IUBMB) classification (1978)</td>
<td>Aliesterases (Est-B) prefer aliphatic aromatic esterases. Assigned Enzyme Commission number 3.1.1.1 to these enzymes.</td>
<td>[36]</td>
</tr>
<tr>
<td>3.</td>
<td>Phylogenetic criteria</td>
<td>Based on sequence similarity of carboxylesterase encoding nucleotide sequences classify them into five families CES 1, 2, 3, 4, 5. Structure and functions. In bacteria, CES are divided into 8 families which were later expanded to 15 families (Table 2).</td>
<td>[7,37,38]</td>
</tr>
<tr>
<td>4.</td>
<td>Based on primary structure comparison</td>
<td>Lipoprotein lipase (L-family), Hormone sensitive lipase (H-family) Cholinesterase (C-family)</td>
<td>[1]</td>
</tr>
</tbody>
</table>

Table 3 Arpigny and Jaegar classification scheme for microbial CES.

<table>
<thead>
<tr>
<th>Family code</th>
<th>Prominent features</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family I</td>
<td>Lipases with six subfamilies</td>
<td></td>
</tr>
<tr>
<td>Family II</td>
<td>GDSL motif with active Ser (S) mostly contain triad conserved at C terminal sequence</td>
<td></td>
</tr>
<tr>
<td>Family III</td>
<td>Family III contain majority of lipids only few are esterases</td>
<td></td>
</tr>
<tr>
<td>Family IV (HSL)</td>
<td>Pentapeptide GDSAG motif with nucleophilic Ser (S) and HGGG W/F/Y motif</td>
<td>[7]</td>
</tr>
<tr>
<td>Family V</td>
<td>GLSMG consensus sequence</td>
<td></td>
</tr>
<tr>
<td>Family VI</td>
<td>GFSQG, GFSNG conserved sequence</td>
<td></td>
</tr>
<tr>
<td>Family VII</td>
<td>GESAG sequence</td>
<td></td>
</tr>
<tr>
<td>Family VIII</td>
<td>Ser-X-X-Lys motif in N-terminal part of enzyme</td>
<td></td>
</tr>
<tr>
<td>Family IX</td>
<td>GYSLG motif</td>
<td>[39]</td>
</tr>
<tr>
<td>Family X</td>
<td>GHSGLG pentapeptide motif</td>
<td>[23]</td>
</tr>
<tr>
<td>Family XI &amp;XII</td>
<td>Majority of lipases are in both the families</td>
<td></td>
</tr>
<tr>
<td>Family XIII</td>
<td>GLSLG pentapeptide motif</td>
<td>[40]</td>
</tr>
<tr>
<td>Family XIV</td>
<td>CHSMG pentapeptide motif with Cys (C) residue in place of Gly (G)</td>
<td>[41]</td>
</tr>
<tr>
<td>Family XV</td>
<td>GLSTG pentapeptide motif instead of common GXSXG motif</td>
<td>[42]</td>
</tr>
</tbody>
</table>

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Family VII

Esterases from this family show similarity to acetylcholiesterase from the eukaryotes and intestine liver carboxylesterase. The sequences show the conserved pentapeptide motif GESAG. The Bacillus subtilis esterase was found to hydrolyze para-nitrobenzyl esters more effectively. The esterase sequences from this family encode proteins of molecular mass 60-65 kDa and are largest known esterases. These enzymes have ability to sterically hindered esters of tertiary alcohol and polyurethanes which are widely used in the industry. The enzymes from this family are characterized from compost metagenomic library [60].
Family VIII
The members belonging to this family show high sequence identity to peptidases and β-lactamases as is true carboxylesterase from Brevibacterium linens IFO12171 [61]. The primary sequence of these enzymes show conserved sequence motif S-X-K at the N-terminal whereas C-terminal of these enzymes also have a conserved sequence motif G-X-S-X-G. The S-X-K motif is conserved in peptidases and β-lactamases also [62-65]. Jeon et al. reported an esterase EstU1 from soil metagenome which has the same active site residue(s) to be involved in ester bond (PNP ester) as well as amide bond hydrolysis (β-lactamases). This unique catalytic action of the enzyme was supported by site directed mutagenesis [27]. The enzymes from this family are reported from different environments including marine sponge, cold adapted and metagenomes obtained from compost and leachate environment [29,66]. Most of carboxylesterases included in this family are from metagenomes. Mokoena et al. referred the esterases from this family as poorly characterized esterases [67]. Est C represents the first member of Family-VIII esterases with the unique β-lactam hydrolytic activity [68].

Family IX
This family came into existence with a characterized thermostable esterase HydS14 obtained from actinobacteria Actinomadura sp. strain S14. The esterase was cloned and expressed for the first time in Pichia pastoris instead of commonly used E. coli as an expression vector. The encoded proteins have a common pentapeptide motif GYSLG [69].

Family X
An esterase EST D from hyperthermophilic Thermotoga maritima is the first characterized member of this family. Proteins are present in monomeric form. It is a second most stable bacterial esterase with a half-life of one hour at 100°C [23].

Family XI and XII
Both these families consist of majority of lipases.

Family XIII
All the members in this family are derived from Bacillus sp. The other members of this family include EstOF4 from Bacillus pseudofermosus. The enzymes are moderately thermostable and are adapted to extreme alkaline environment (pH 11). The Proteins in native form are dimers and are hydrophobic [40].

Family XIV
This family came into existence with the thermostable esterase EstA3 from Thermoanaerobacter tengcongenesis MB4. The enzyme sequence contains a pentapeptide motif CHSMG with Cys (C) residue at the first position [41].

Family XV
The enzymes from this family contain ‘GLSTG’ a distinct pentapeptide motif which is quite different from the common GXSXG motif found in most of the esterases. The esterase(s) from Geobacillus thermoleovorax CCR11 in this family show resistance to temperature, pH and organic solvents and is therefore found to be very amenable in the processes like oleochemical biotransformations, pharmaceuticals, cosmetics, organic synthesis and biodiesel production [42,70,71].

Applications of CEs
The extensive uses of these enzymes result in the search of novel sources of CEs with functional values suited well for their industrial applications. These enzymes are well known for their role in green chemistry.

Detoxification reactions and environmental monitoring
Insecticides can be degraded by chemical as well as microbial interventions. Artificially created pesticides are often ester compounds. Microbial breakdown and environmental reactions like hydrolysis, photolysis and oxidation with minerals are some of the reactions that convert pesticides into nontoxic or less toxic compounds. Microbial breakdown is least harmful, safe, ecofriendly and economic method exploiting the application of CES (Figure 2). Pyrethroids and malathion are organophosphorus insecticides having harmful effects on human beings and other higher organisms [72-74]. Enzymes from Alicyclobacillus tengcongenesis degrade malathion quite efficiently. Malathion also acts as a sole carbon source for the bacterial species grown in minimal salt medium such as Bacillus licheniformis helps in the bioremediation of soil that is polluted with malathion [75,76]. The degradation of malathion by Brevibacillus sp. and Bacillus cereus has also been reported [77]. Toxicity caused due to OPs results in the inhibition of acetylcholinesterases (a neurotransmitter) resulting in the disruption of the nervous system in the rats and other pests due to formation of neurotransmitter acetylcholine. CEs bind stochiometrically to these chemicals. Cleavage of pyrethroids by CEs is also one of the detoxification pathways in mammals and insects which possess isomer selectivity. Carboxylesterase gene(s) from Bacillus cereus SM3, Aspergillus niger ZD11, Nethotettix cincticeps and mouse liver microsomes degrade carboxylester linkages in pyrethroids have been successfully purified. The CE genes from liver microsomes and those from Klebsiella sp. strain ZD112 were cloned and functionally expressed [72].

Endobiotic compounds processing
CEs play important roles in endobiotic processing of hydrophobic compounds e.g., cholesterol, which is an important constituent involved in maintaining the structural integrity of plasma membrane. Its excess causes a harmful effect though its normal quantity plays a vital role in cholesterol homeostasis in the animals. CEs transform fatty acid at 3rd position of the cholesterol to form cholesteryl ester.

Biocatalysis
Besides detoxification reactions, CEs are involved in bio catalysis, a chemical reaction in which one or more enzymes catalyze the chemical reaction, which find potential applications in synthetic chemistry.
Synthesis of optically active compounds

CEs are also involved in bio catalysis especially in organic compounds synthesis. Their use in ‘White Biotechnology’ for the synthesis of industrially important chiral compounds is increasingly important in pharmacology. The drugs that commonly enter in the market consist of one of its either of two isomers. Due to their high region-stereo specificity, CEs are used in the synthesis of optically pure compounds. One of such compounds i.e., carboxylesterases NP (Naproxen) from Bacillus subtilis Thai I-8 was characterized as a very effective enantioselective biocatalyst for NSAID. Naproxen is non-steroidal, pain killing drug with only one of its optical isomer that is commonly used [77]. The CEs show modest selectivity towards chiral alcohol as compared to carboxylic acids. It is produced as an intracellular protein with a molecular weight of 32 kDa, with optimum pH of 8.5-10.5 and optimum temperature between 35 to 55°C. Besides carboxylesterase NP, 2-arylpropanoic acid is produced with high enantioselectivity [1°]. An esterase from Arthrobacter globiformis is used in the chemical synthesis of (+)-trans-(1R, 3R) - chrysanthemic acid, which is an important precursor of pyrethrin insecticides [78].

Food industry

As food additives, enzymes act as important means to enhance the flavor, texture and taste of the foods. Esterases are commonly used in making cheese and other milk derivatives and their important role is considered in improving aroma, taste and texture of many dairy products. Esters are also important for the aroma of fermented beverages like wine with ethyl acetate as the most common ester in wine due to its ready formation from ethanol and acetic acid. High reactivity of a primary alcohol and its low concentration gives specific fruity character to the wine [50]. The first CE cloned and characterized from wine was associated with bacterium Oenococcus oeni. Esters from Pseudomonas fragii and Ferribidobacterium nodusum Rt 17 B-1 are responsible for fragrance and fruit-like flavours. Free fatty acids in cheese are important for flavor. CEs play important role in apple flavor and their biosynthesis increases with the fruit ripening hormone ethylene. During early stages of fruit development these enzymes are expressed at low levels. At maturity their expression level increases [79]. These enzymes also play role in the chemical modification of many drugs and produgs like antiplatelet drug aspirin, clopidogrel and drugs like delapril, imadapril, and temocapril which are acetylcholiesterase inhibitor and an antitumor drug irinotican [80].

Cancer gene therapy

CEs have been implicated in the activation of a number of anticancer agents like CPT-11, irinotican [7-ethyl-10-[4-(1-piperidino)-1-piperidino] and carboxyoxycamptothecin, which are active against a broad range of cancers including colorectal and cervical cancer. Anticancer effect of CPT-11 is dependent on its conversion to ethyl camptothecin, which is catalysed by one or more CEs particularly CE-2. These enzymes play role to activate prodrugs in vivo and generate effective anticancer agents in highly selected target site at the surface of tumor cells or inside them CEs are targeted to tumor sites with hybrid monoclonal antibodies or CDNA encoding a CE is targeted to tumor cells by viral vector. This therapeutic strategy releases anticancer drug ethyl camptothecin in the vicinity of tumor cells [79], thus killing them effectively.

Plastic depolymerization and recycling

Microbial CEs are effective biocatalysts and can hydrolyze various polyesters effectively and play an important role in plastic depolymerization like polylactic acid (PLA). Recycling of PLA is required which produces CO₂ [81,82]. Tchigvintsev et al. reported
that metagenomic esterases have ability to hydrolyze many polyester substrates including PLA [15].

Conclusion
Ecofriendly nature and various other applications of CEs such as their role in drug metabolism, food industry and clinical use prove them to be beneficial. Because of their use in xenobiotic compounds processing (especially OPs) and insecticide-resistance in microorganisms, these enzymes are emerging as the basis of various bioremediation methods to lessen the effect of xenobiotic environmental pollutants. Further studies on these enzymes will not only help to explore new strategies of pollution control methods but will also provide diverse industrial applications for human welfare.

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Conflict of Interest
None of the authors have conflict of interest, financial or otherwise among themselves or with the parent institute.
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